Impact of Medicinal Plants Incorporated Product on Patients Suffering from Periodontitis

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Annexure

Abbreviations used in the study

WHO - World Health Organization

AAP - American Academy of Periodontology

CAD - Coronary Artery Disease

CVD - Coronary Vascular Disease

NIH - National Institute of Health

GRAS - Generally recommended as safe

FDA - Food and Drug Administration

COPD - Congestive Obstructive Pulmonary Disease

OPD - Out Patient Department

1.0 Introduction

1.1 Periodontitis

Gingiva, alveolar bone, periodontal ligament and cementum of root compose Peridontium. Peridontium is the supporting structure of tooth as it surrounds the teeth and keeps teeth tightly in their place (Antonio & Bosshardt, 2006).

Definition given by **American Academy of Periodontology** "Periodontitis is chronic inflammation of the tooth supporting structure i.e. Peridontium". It mostly starts with the inflammation of gums called gingivitis. When inflammation confined only in margins of the gingiva, it is called gingivitis and when it reaches proper gingiva and alveolar mucosa lead to loss of periodontal ligament along with alveolar bone causes destruction of tooth supporting structure, it is called Periodontitis (AAP, 2000).

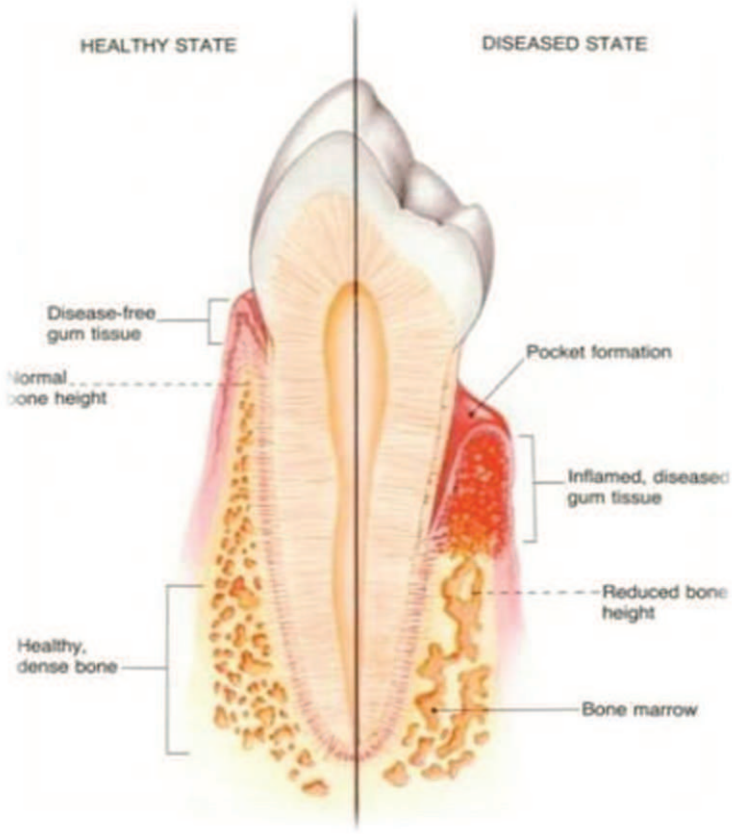

Figure 1.1 – Healthy Peridontium vs Periodontitis
Source –Placyk, 2016

1.1.1 Types of chronic periodontitis (Armitage, 2004)

A) Localized chronic periodontitis

If periodontitis present in <30% teeth, It is termed as localized periodontitis.

B) Generalized chronic periodontitis

If periodontitis present in >30% teeth, It is termed as generalized periodontitis.

1.1.2 Prevalence of Periodontitis

Globally

Periodontal infection is the major cause of tooth loss. According to WHO 10% to 15% of world population has severe periodontitis. Prevalence of periodontitis is less approximately 10% in countries like America, Japan, Australia, China, Malaysia, Norway, Brazil and Denmark. Prevalence of periodontitis is more than 20% in India, Bangladesh, Canada, Chile (WHO, 2012).

India

Jacob et al. (2011) studied the prevalence of Periodontal Disease in India and concluded that only two surveys have been done in India. One by Dental council of India and other by Government of India in association with World health organization. Survey done by Dental Council of India in 2004, states that moderate to severe periodontitis 21.8% and 18% present in older age people whereas in younger group percentage was 17.5% and 7.8%. Government of India in association with WHO also conducted a survey in 2004, in different states Arunachal Pradesh, Uttar Pradesh, Rajasthan, Delhi, Madhya Pradesh, Orissa, Pondicherry. High prevalence of periodontal disease approximately was 100% found in Orissa and Rajasthan in younger people as well as in older people. Loss of attachment one of the marker of periodontal disease was highly prevalent in Maharashtra about 78% in younger people and 96% in older people, followed by Orissa and Delhi. Rest of states have prevalence rate of 15% to 35%.

Agarwal et al. (2014) states India is an agricultural country. 70% of the population in India is rural. Periodontal health is better in urban area then rural area because literacy rate is higher in urban area and also there is more health care providers (Dentist). Most of the dental colleges are

in urban area. Students graduating from dental colleges also like to practice in city. Although Government and dental colleges organize camps for creating awareness and treatment in rural area but because of illiteracy and negligence towards oral health as well as high cost treatment coupled by conveyance problem of rural population for the city. Thus, Prevalence of periodontitis is more in rural area.

1.1.3 Diagnosis of chronic periodontitis

A) Sign and Symptoms of Periodontitis (Friedwald et al., 2009)

- Halitosis (Bad breath)
- Swollen gums that bleed
- Sensitivity in teeth
- Difficulty in Chewing
- Mobile teeth
- Gum recession

B) Clinical Diagnosis

- Red swollen gingiva
- Rolling of margins of gingiva
- Loss of stippling surface
- Bleeding on probing
- Increases probing depth which divides periodontitis into 3 categories

i. **Mild –** 2-3 mm clinical attachment loss of gingiva measured by CPITN probe from cemento-enamel junction.

ii. **Moderate** - 3-4 mm clinical attachment loss of gingiva measured by CPITN probe from cemento-enamel junction.

iii. **Severe** - 5mm or greater than loss of clinical attachment of gingiva measured by CPITN probe from cemento-enamel junction.

1.1.4 Etiology of periodontitis

(A)Dental plaque

After brushing a thin biofilm (dental plaque) by salivary action is formed on teeth. Soon in this biofilm bacteria start colonization. They are gram positive aerobic bacteria. Some bacteria are pathogenic some are non-pathogenic. If patient ignores oral hygiene practices, thickening of biofilm starts due to colonization of bacteria and also due to deposition of calcium and other minerals present in saliva, this causes formation of calculus (tartar). In this calculus, more and more bacterial growth occurs and now it is not only confined in marginal gingiva but also starts invading below in proper gingiva. There is no oxygen supply so now microbial flora becomes anaerobic. Gram negative anaerobic bacteria start to colonize and release toxins. In response to toxins, immune system becomes active. Leucocytes as well as rise in inflammatory markers of the body in response to bacterial toxins causes splitting of gingiva, loss of periodontal ligament and reabsorption of bone leading to formation of periodontitis (Hasan & Palmer, 2014).

The gram negative anaerobic bacteria which provoke periodontitis is

- Porphyromonas gingivalis
- Prevotella intermedia
- Tannerella forsythia
- Treponema denticola
- Aggregatibacter actinomycetemcomitans

(B)Other etiologic factors are (Dyke & Dave, 2005)

- Smoking
- Diabetes
- Hormonal changes
- Genetic susceptibility
- Certain medications
- Malocclusion
- Faulty eating habits
- Lack of exercise
- Stress
- Age

1.1.5 Periodontitis as a risk factor for Non-communicable diseases

Bacteria that colonize in gingiva start releasing endotoxins. At first gingival epithelium act as a barrier but due to bacterial toxins soon it become ulcerated and then bacteria and their toxins come in connect with underlying connective tissue and blood capillaries that lead to entry of bacteria in circulation during brushing and eating of food. As bacteria and their toxins (cytolytic enzymes and lipopolysaccharide) entered in circulation, Individual's immune system responds by production of inflammatory cells and their mediators like tumor necrosis factor (TNF-α),interleukin (IL)-1β, prostaglandin E2 and γ interferon lead to change in body's normal phenomenon causing development of disease or increasing the severity of systemic diseases. Thus, bacteria directly or indirectly by toxins affects all body system of host (Igari et al., 2014).

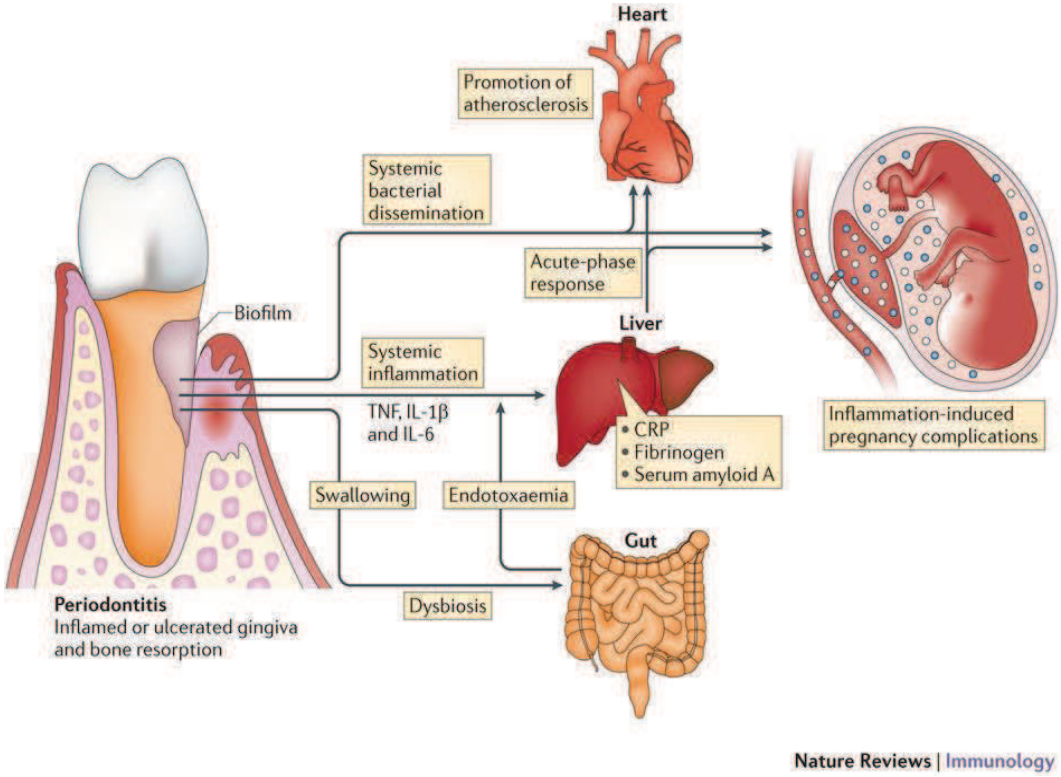

Figure 1.1.5 – Relationship between periodontitis and non-communicable diseases

Source –Hajishengallis, 2015

1.1.6 Prevention and Treatment of periodontitis (IDA, 2016)

- ❖ Quit tobacco use, or reduce the amount smoked, or not start the habit
- ❖ Brush twice a day
- ❖ Use of mouthwash and floss regularly

- Visit to Dentist in every 6 month
- Scaling and root planning
- Antibiotics
- Make healthy food choices
- Be physically active
- Control glycaemia
- Phytotherapy in treatment of periodontitis (Lokhande et al., 2006; Shama et al., 2014).

1.2 Importance of medicinal plants in preventing and curing diseases

Due to increased rate of oral diseases and use of antibiotics to cure, bacteria became resistant to these antibiotics as well as these drugs **can alter oral microbiota and have undesirable adverse effects such as vomiting, diarrhea and tooth staining**. These antibiotics are costly specially in developing countries, also these antibiotics cause adverse effects. Thus, there is need for alternative treatment for prevention and treatment which have no or less side effects, effective and also cost-effective.

Thus, natural phytoconstituents isolated from plants used as traditional medicines are considered as good alternative (Singh et al., 2016).

Luqman et al (2014) states that medicinal plants are natural source of vitamin and minerals which provide nourishment to the body and also they contain some active phytoconstituents which have healing properties as well as anti-inflammatory property. Phytoconstituents are those biologically active substances found naturally in plants that is responsible for its color, smell and most important it have medicinal properties that cure diseases (Ambwani, 2014). Medicinal plants have no or less harmful effect on body then allopathic treatment. They are easily available. Herbs with medicinal properties improve the immune system, rectify disease rather than suppress (Luqman et al, 2014).

Example- **Eugenol** present in Tulsi is a phytoconstituent which is potent immunomodulator (Ministry of AYUSH, 2011)

Cinnamaldehyde present in Cinnamon is phytoconstituent which have anti-inflammatory activity (Jamal, 2009)

1.3 Selection of Medicinal Plants and their active Phytoconstituents

Extensive search is done on internet, journals, articles and reports for Medicinal plants that cure periodontal disease. List of medicinal plants is prepared out of which those medicinal plants were selected which were commonly available and found to be effective in curing and preventing periodontitis in their in-vitro and clinical trials. The plants selected are *Tulsi, Amla, Stevia, Cinnamon* and *licorice root*.

1.3.1 Tulsi

Tulsi is also called holy Basil. It is cultivated in almost every house in India because of its divinity written in all Purans. Mostly leaves are used in making ayurvedic preparations.

Types of Tulsi– (Das &Vasudevan, 2006)

- Ocimum sanctum Linn - Tulsi
- Ocimum gratissimum – Ram Tulsi
- Ocimum basilicum Linn – Dulal Tulsi
- Ocimum kilimandscharicumGuerke – Camphor Basil
- Ocimum americanum Linn – Hoary Basil
- Ocimum micranthum Wild

Among all of species Ocimum sanctum has most therapeutic properties.

Medicinal properties of Tulsi (Prakash & Gupta, 2005)

- Antimicrobial
- Antioxidant
- Anti-inflammatory
- Hypoglycemic
- Hypolipidemic
- Immunomodulatory
- Anti-ulcer
- Anti-stress
- Chemo protective and radioprotective

Active Phytoconstituent of Tulsi -

Joshi and Hoti (2014) analyzed the phytoconstituents of *Tulsi* in laboratory. They found total 26 constituents in *Tulsi* extract, out of which **80 % are eugenol and methyl eugenol**.

Other constituents are β-caryophyllene, borneol, germacrene D and α-copaene, sesquiterpene hydrocarbons, monoterpines.

Dev et al. (2011) also analyzed the chemical composition of different extract of *Ocimum* leaves. The major constituents were found in essential oil are **eugenol (61.76%),** isopropyl palpitate ,α-cubene , 3dihydroxy propyl elaidate ,1-methyl-3-(1-methyl) benzene ,2-methoxy4-(1-propyl) phenol ,vanillin , 4-diethyl benzene (1.03%), hexadecanoic acid methyl ester and [2-methyl-4-(1-propyl) phenoxy] silane.

1.3.2 ***Cinnamon***

Das Manosi et al (2013) states that *Cinnamon* is a common herb used in almost every kitchen as a spice or flavoring agent. Inner bark of the tree is dried and used as spice as well it has medicinal properties.

Types of cinnamon - (Maheshwari et al., 2013)

There are two common types of Cinnamon.

1) "True Cinnamon" from the Cinnamon tree which is native in Sri Lanka (from the genus Cinnamomum verum or Cinnamomum zeylanicum)
2) "Cassia" (Cinnamomum Cassia) which is being commercially cultivated in China, Indonesia, Vietnam and other Southeast Asian countries.

It′s widely accepted that Cinnamon from Sri Lanka is of better quality between the two

Active Phytoconstituent of Cinnamon (Vangalapati et al., 2012)

- **Leaves** - Cinnamaldehyde: 1.00 to 5.00% Eugenol: 70.00 to 95.00%
- **Bark** - Cinnamaldehyde: 65.00 to 80.00% Eugenol: 5.00 to 10.00%
- **Root bark** - Camphor: 60.00%
- **Fruit trans** -Cinnamyl acetate (42.00 to 54.00%) and caryophyllene (9.00 to 14.00%)
- **C. zeylanicum buds** - Terpene hydrocarbons: 78.00% alpha-Bergamotene: 27.38% alpha-Copaene: 23.05% Oxygenated terpenoids: 9.00%
- **C. zeylanicum flowers** - (E)-Cinnamyl acetate: 41.98% trans-alpha-Bergamotene: 7.97% Caryophyllene oxide: 7.20%

Medicinal Properties of Cinnamon (Rao & Gan, 2014)

- Anti-oxidant
- Anti-inflammatory
- Antimicrobial
- Antibacterial
- Analgesic

Cinnamon is commonly used in rheumatoid arthritis, periodontitis, and toothache, lowering cholesterol level, curing respiratory diseases, fighting infection and has healing properties(Vangalpati et al., 2012).

1.3.3 Amla

Meena, Singh & Rao (2010) states that*Amla* (Emblica officinalis) also called as Indian gooseberry is one of the rich sources of vitamin C and it is used in various preparations like Chyavanprash, Triphlachuran.

Active phytoconstituent of Amla

- Tannins (28%) in form of **Emblicanin A and B**,
- Other constituents are **phyllemblin,** linolenic, linoleic, oleic, stearic, palmitic and myristic acid.

Medicinal properties of Amla (Bhide et al., 2014)

- Potent antioxidant
- Antibacterial
- Hypoglycemic
- Hypolipideamic
- Expectorant
- Spasmolytic
- Immunomodulatory
- Antipyretic
- Analgesic
- Cytoprotective
- Diuretic
- Laxative
- Carminative
- Stomachic

- Antitussive
- Gastroprotective

Additionally, it is useful in memory enhancing, ophthalmic disorders. It is also helpful in neutralizing snake venom and as an antimicrobial.

1.3.4 Liquorice (Glycyrrhzia glabra)

Damle (2014) states that, in north India *Liquorice* is commonly known as Yashtimadhu or Jothimadh. As name states it is sweet herb, mainly root of plant is used for therapeutic purpose.

Active phytoconstituent of Liquorice (Isbrucker& Burdock, 2006)

- Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) 10 to 20%.
- Starch (30%)
- Other constituent are polysaccharides, gums, mucilage, amino acids, triterpene saponin, flavonoids, mineral salts, asparagines, tannins, glycosides, resins, sterols, and volatile oils.

Medicinal Properties of Liquorice ((Isbrucker& Burdock, 2006)

- Ant-allergic
- Anti-inflammatory
- Antibacterial
- Antipyretic
- Hepatoprotective
- Analgesic
- Antioxidant
- Immuno-stimulatory
- Hypoglycemic
- Cardioprotective

1.3.5 Stevia *(Stevia rebaudiana)*

Mandal & Madan (2013) states that *Stevia* is non-caloric natural sweetener possessing antioxidant and immune-enhancer property. It is 300 times sweeter than sucrose.

Species of Stevia (Gupta et al., 2013)

There are around 150 species within the Stevia family. Most common are -

Stevia dianthoidea, Stevia Phlebophylla, Stevia anisostemma, Stevia bertholdii, Stevia crenata, Stevia enigmatica, Stevia eupatoria, Stevia lemmonii, Stevia micrantha, Stevia ovata, Stevia plummerae, S. rebaudiana, Stevia salicifolia, Stevia serrata and Stevia viscida.

All plants of stevia are sweet but rebaudiana having the highest sweetness levels. It is also known as sweet herb, sweet leaf, honey leaf, candy leaf and honey yerba

Active phytoconstituent of Stevia (Mandal & Madan, 2013)

- Stevioside
- Rabudioside
- Dulcosides
- Steviolebusides
- And other phytoconstituents are flavonoids, phenolic compounds, and minerals and vitamins.

Medicinal properties of stevia (Madan et al., 2010)

- Antihypertensive
- Anti-hyperglycemic
- Antioxidant
- Anti-cariogenic
- Anti-inflammatory
- Immunomodulatory
- Antiviral properties

Conclusion - Since periodontitis is a oral disease that is confined in mouth but have systemic effects by means of bacteria and their toxins ingestion during brushing, chewing and ulceration due to toxins in the gums lead to development or increase in the severity of systemic diseases. Periodontitis can be prevented and cured by maintaining good oral hygiene habits as well as use of oral products like mouthwash, dental gel, toothpaste and allopathic or herbal candy which have antibacterial as well as wound healing effects.

Thus, present study is planned to develop medicinal plants incorporated product for prevention and cure of periodontitis to improve oral health as well general health by inhibiting the bacterial growth in mouth and their systemic ingestion during brushing, chewing or via ulceration due to bacterial toxins.

Table 2.0 - Review of Literature

Intervention studies of selected medicinal plants

Medicinal Plant	Researcher	Study	Conclusion
Tulsi	**Gaur et al. (2015)**	A randomized controlled clinical trial on 30 patients with 2 ml of extract irrigated each day, thrice with interval of 15 days for 4 weeks	It was concluded that Tulsi extract was equally effective as chlorhexidine.
	Gupta et al. (2013)	Placebo controlled, triple blind, randomized clinical trial on 108 patients divided in 3 groups. Group 1 (36 patients) was given 4% Ocimum sanctum mouthwash and instructed to use 10 mL twice a day for 30 days. For group 2 and 3, chlorhexidine and saline water was given.	After intervention, it was concluded that There was progressive decrease in the plaque and gingivitis score in Ocimum sanctum and chlorhexidine groups. Chlorhexidine group showed maximum decease as compared to Ocimum sanctum group but difference was not statistically significant
Licorice root	**Sharma et al. (2016)**	Invitro study of Glycyrrhizin glabra extract on periodontal pathogens.	In the study, 67% ethanolic extract of Glycyrrhizin glabra showed antimicrobial activity toward periodontal pathogens

Medicinal plant	Researcher	Study	Conclusion
Licorice root	**Farhad et al. (2013)**	Randomized clinical trial on 39 patients divided in 3 groups. 20 mg doxycycline prescribed to 1 group, 400 mg *licorice* (380 mg *licorice*, 8-12 mg Glandenic acid) and 3rd groups is on placebo capsules	After the clinical trial, they concluded that doxycycline and *licorice* was equally effective in decreasing periodontal inflammatory markers.
Cinnamon & Amla & Tulsi	**Sharma et al. (2015)**	Developed a polyherbal tablet containing Azadirachta indica, **Ocimum tenuiflorum**, Caryophyllus aromaticus, **Cinnamomum zeylanicum,** Quercus infectoria, **Emblica officinalis**, Terminalia chembula and Acacia Arabica ethanolic extract. For control group tetracycline 100mg was used	After intervention, it was concluded that the zone of inhibition of all extract is less as compared to standard antibiotic Tetracycline
Cinnamon & Licorice	**Suchetha & Bharwani (2016)**	Designed clinical trial to test commercially available herbal product Periocare containing Licorice, *Cinnamon* and *Piper nigrum*. They selected 75 volunteers for the study and divided into 3 groups (25 subjects in each group). 1st group (scaling+ periocare), 2nd group (Periocare), 3rd group only scaling.	After clinical trial 1st group shows significant reduction in markers of periodontitis

2.0 Review of literature

Relevant literature has been collected and presented in following headings to critically analyze the gaps in knowledge

i) Introduction

ii) Safe daily intake and safety concern of selected medicinal plants

iii) Intervention studies of selected plants in preventing and curing periodontitis

2.1 Introduction

In today's scenario, non-communicable diseases are more prevalent than communicable disease because of spread of urbanization, change in food taking pattern and increase in sedentary lifestyle (Fustur, 2014).

In past few years, Periodontal disease emerged as one of the risk factor for developing non - communicable diseases. Various studies conducted for identifying the association between periodontitis and systemic diseases. Some studies support periodontitis as a risk factor and some studies not. Hasan and Palmer (2014) summarized all longitudinal and case control studies done for finding association between periodontitis and systemic diseases and mechanism associated behind this, and concluded that periodontitis is a risk factor for developing systemic diseases as bacteria continuously colonizes and growing in gingiva also releasing toxins causing bacteremia and increase in inflammatory markers of immunity such as interleukins, cytokines, C- reactive protein and also defense cells like fibroblast and macrophages which increase oxidative stress in the body that cause damage to body tissues lead to development or progression of non – communicable diseases.

Thus, by curing and preventing periodontitis reduce the inflammatory markers of the body which increase the oxidative stress (Jeffcoat et al., 2014).

Apart from the popular treatment by antibiotics and commercially available mouthwash now a days research studies are conducted for checking the efficacy of herbs in prevention and treatment of diseases. Herbs are rich in nutritional content and have medicinal value. They have active

phytoconstituents and also Herbs are natural source of minerals and vitamins possessing anti-inflammatory and antibacterial properties. They also enhance immunity level of the body, have no ill effects on body, easily available and cost effective method for treating diseases (Kamboj, 2000).

2.2 Safe daily intake and safety concern of selected medicinal plants

Table -2.2 - Acceptable Safe daily intake of Selected Medicinal Plants

Common Name	Botanical Name	Acceptable safe daily intake	Sources
Tulsi	Ocimum sanctum	1 gm/day	Ministry of AYUSH, 2011
Amla	Emblica officinalis	868 mg/day	FDA, 2013
Stevia	Stevia rebaudiana	4mg/kg/bw	FDA, 2010
Cinammnon	Cinnamomum Verum	6 g/day	NIH, 2012
Liquorice root	Glycyrrhzia glabra	100 mg/day	European commission, 2003
Isomalt	Isomalto-Oligosacchride	2.5-5.0 gm/dose 30 gm/day	FDA, 2005

**Isomalt (Isomalto-Oligosacchride)* will be used in place of sugar in case of candy development.

2.2.1 *Isomalt(Isomalto-Oligosacchride)*

FDA, 2005 in its GRAS notice states that *Isomalt* is a short chain non-digestible carbohydrate derived from starch of cereal crops like wheat, barley, potato used generally as sweetener in food industry. It is low in calorie; act as prebiotic because it supports the growth of beneficial bacteria present in colon with least possibility of any adverse reaction.

It is 60% to 70% sweeter that of sucrose used in all kind of beverage, dairy, sweet and dessert making industries. It does no change blood sugar level or insulin level thus used by diabetic patients. Safe daily intake is up to 30gm/day. At 15gm/day it does not effect on the blood lipid level and glucose absorption.

2.2.2 *Stevia (Stevia rebaudiana)*

FDA, 2010 in its report states that *Stevia rebaudiana* is a non-nutritive sweetener with the acceptable daily intake of 0-4 mg/kg/body weight/day. It is used as a sweetener in non-alcoholic

beverages, baking, confectionaries, dairy, processed food and fruit juices, medical food. In clinical trials it does not reflect any genotoxicity. It digested and absorbed in intestine. As per the FDA no hepato-toxicity and no any side effects were found within the consumption limit.

2.2.3 *Amla (Emblica Officinalis)*

FDA, 2013 in its GRAS notice states that *Amla* is used as flavoring agent and it is potent antioxidant used in processed fruits and fruit juices, non-alcoholic beverages, with a maximum daily intake of 868mg/person/day. It cannot be used for infants and toddlers and also in meat and meat products. *Amla* is used in preparations of chywanprash, jam, chutneys, pickles, extract of *Amla* used as dietary supplement for vitamin c in a dose of 1gm/day. Because of its sour taste it is used as flavoring agent. *Amla* in clinical trials and vitro studies does not show any side effects, toxicity. It is safe to use within its limit.

2.2.4 *Tulsi (Ocimum Sanctum)*

Ministry of AYUSH, 2011 supported a project conducted by AIIMS (Mohapatra et al., 2011) at Immunomodulatory effects of *Tulsi* on healthy subjects. In project researcher conducted a double blind randomized clinical trial in cross over format on 24 subjects. *Tulsi* dose in 300mg capsules were given to experimental subjects for 3 months then all vital signs and parameters were checked and left for 1 month for washout period. Again all parameters checked and 3 months of Tulsi dosing were provided to them. No adverse effect of Tulsi was found on experimental group. No significant changes in biochemical parameters of body although it slightly reduces the cholesterol level.

2.2.5 *Cinnamon (Cinnamomum verum)*

NIH, 2012 states that *Cinnamon* is a popular spice used as flavoring agent in food also has medicinal properties. It is used in controlling diabetes, bronchitis, GIT problems. It's safe daily intake is 6gm/day for 6 weeks. One of its constituents has blood thinning property so it can't be used in people who are taking blood thinners.

2.2.6 *Licorice (Glycyrrhzia glabra)*

FDA, 2015in its GRAS notice states that *Licorice* is sweeter than sucrose used in food industry as food additive. At high doses it shows pseudohyperaldosteronism. It is hypertensive in high doses. It does not have any toxic effect on the body.

After selection of medicinal plants and their safe daily intake next step is reviewing the literature for intervention studies/ clinical trials related to these plants.

2.3 Intervention Studies of Selected Medicinal Plants in preventing and curing Periodontitis

2.3.1 *Tulsi (Ocimum sanctum)*

Gaur et al. (2015) conducted a randomized, controlled clinical trial on patients suffering from periodontitis.30 patients were selected from the OPD of Department of Periodontology and Oral Implantology, I.T.S Dental College, Muradnagar, Ghaziabad.

The experimental procedures were undertaken with the understanding and written informed consent of the patient and the study was approved by the ethical committee of the institution.

A randomized, controlled clinical trial was conducted to compare the efficacy of scaling and root planning with pocket irrigation with Ocimum Sanctum versus Chlorhexidine in patients diagnosed with chronic periodontitis.

Inclusion criteria

- Patients of both the gender between ≥ 28 years of age, diagnosed with chronic periodontitis
- Periodontal pocket measuring ≥ 5 mm
- Patients who were nonsmokers or smoking < 5 cigarettes /day were included in the study

Exclusion criteria

- Subjects on antibiotics for last three months
- Patients who undergone periodontal therapy in the past six months
- Patients with systemic diseases

- Smokers
- Alcoholics
- Patients with less than 8 teeth in the oral cavity.

30 patients were randomly divided into two groups. In Group A, 15 sites were treated with scaling and root planing with intra pocket irrigation with Ocimum Sanctum and in Group B, 15 patients were treated with scaling and root planing with intrapocket irrigation with 0.2% chlorhexidine was done. Each patient was irrigated with 2 ml of solution, thrice at 15 minutes of interval for 30 days. Thus, after 4 weeks of intervention results obtained conclude that Ocimum sanctum was equally effective in reducing periodontal indices as chlorhexidine. The results demonstrated a significant reduction in all clinical parameters in both groups.

Gupta et al. (2013) conducted a placebo controlled, triple blind, and randomized controlled clinical trial placebo-controlled, (patients, examiner, statistician was blinded to the allocation of mouthwash in respective groups); three group parallel study was conducted in the department of public health dentistry on volunteered medical students of Teerthanker Mahaveer Medical College and Research Centre. Protocol was approved by the Institutional Review Board (IRB) of Teerthanker Mahaveer University. All subjects signed an IRB approved consent form.

Inclusion criteria

- No history of any dental treatment
- No antibiotic or anti-inflammatory drug therapy for the past 3 months
- Participants with good general health, a baseline plaque score greater than 1.5 and a baseline decayed, missing and filled teeth (DMFT) index of 3 to 5 were included in the study.

Exclusion criteria

- Students with any history of systemic diseases/conditions
- Fibrotic gingival enlargement
- Smokers
- Those volunteers who had used antibiotics or mouthwash for 5 consecutive days or corticosteroids in the past 30 days were excluded from the study

- Those subjects who had a history of sensitivity to any mouthwash
- Used removable prostheses or an orthodontic appliance
- Those who had undergone professional measures to remove plaque and calculus in the past 15 days
- Did not give consent for clinical trial were excluded.

A total of 108 volunteers were randomly allocated into the three study groups through computer-generated random numbers. Random allocation of mouth rinses using the lottery method was done. Individuals were identified by code numbers throughout the study.

- Group 1 (n = 36) was given 4% Ocimum sanctum mouthwash and instructed to use 10 mL twice a day for 30 days.
- Group 2 (n = 36) was given 0.12% Chlorhexidine and instructed to use 10 mL twice a day for 30 days.
- Group 3 (n = 36) was the placebo control group and saline water was given as the mouthwash.

Students were instructed to rinse their mouth with 10 mL of mouthwash twice daily once post breakfast and other post lunch for 30 days for 60 sec and not to rinse with water thereafter. Data were collected three times in 30 days

For assessing plaque and gingivitis, clinical measurements for plaque and gingivitis were taken at baseline (prior to the study), at 15 days and at the end of the study (4 weeks).

There was progressive decrease in the plaque and gingivitis score in Ocimum sanctum and chlorhexidine groups. Chlorhexidine group showed maximum decease as compared to Ocimum sanctum group but difference was not statistically significant.

2.3.2 *Licorice root (Glycyrrhzia glabra)*

Salari et al. (2003) checked minimum inhibitory concentration of enoxolone (constituent of *licorice* having antibacterial activity) in laboratory. Peridontittis causing bacteria isolated from patient's mouth by sterile strips and diffused in agar medium at controlled temperature. Different inhibitory concentration determined. They find that MIC of enoxolane is 16mg/ml and at this concentration it is effective in controlling bacterial growth.

Farhad et al. (2013) conducted a clinical trial for testing the efficacy of doxycycline and *licorice* on inflammatory markers of periodontitis present in gingival cervicular fluid. 39 subjects were selected for the study randomly divided in 3 groups; 20 mg doxycycline prescribed to 1 group, 400 mg *licorice* (380 mg *licorice*, 8-12 mg Glandenic acid) and 3 groups is on placebo capsules. After the clinical trial, they concluded that doxycycline and *licorice* was equally effective in decreasing inflammatory markers.

Sharma et al (2016) conducted a invitro study of Glycyrrhizin glabra extract on periodontal pathogens. In this study, 67% ethanolic extract of Glycyrrhizin glabra showed antimicrobial activity toward periodontal pathogens. Although very good results were obtained against primary plaque creating bacteria and periodontal pathogens, but the potency shown was not for longer duration due to Zone of inhibition reduced from 24 to 72 hour, this may have been due to overcoming of the antimicrobial effects of herbal plant extracts by the bacteria present over longer duration, or it may have also due to degeneration of phytochemicals by toxins released by bacteria over period of time.

2.3.3 *Amla (Emblica officinalis)*

Amla is a potent natural antioxidant due to its high Vitamin C content and also it has active phytoconstituents such as Emblicanin. It naturally restores oral health, heal the inflamed tissue and enhance the development of lost connective tissue. It is used as extracts or in form of capsules taken orally for long term advantage (Singh &Purohit, 2011).

2.3.4 *Cinnamon (Cinnamomum verum)*

Suchetha and Bharwani (2013) designed clinical trial to test commercially available herbal product Periocare containing Licorice, *Cinnamon* and *Piper nigrum*. They selected 75 volunteers for the study and divided into 3 groups (25 subjects in each group). 1st group (scaling+ periocare), 2nd group (Periocare), 3rd group only scaling. After clinical trial 1st group shows significant reduction in markers of periodontitis although 2nd group also showed the reduction but not much as with scaling and periocare together.

Sharma et al (2015) developed a polyherbal tablet containing Azadirachta indica, **Ocimum tenuiflorum**, Caryophyllusaromaticus, **Cinnamomum zeylanicum,** Quercus infectoria,

Emblica officinalis, Terminalia chembula and Acacia Arabicaethanolic extract against plaque causing agents. Extracts of all herbs was made and evaluated for their color, taste odor and active phytoconstituent. All plants were having Phenols, Tannins, Steroids, Alkaloids, Glycosides and Flavonoids as active phytoconstituents. For control group tetracycline 100mg was used. The zone of inhibition of all extract is less as compared to standard antibiotic Tetracycline. A polyherbal tablet was made from dried extracts and intervention was done on experimental group.

After reviewing the existing literature, it is concluded that time to time study was done to see the impact of medicinal plant on periodontal pathogens. Active phytoconstituent of medicinal plants effectively controlled the growth of periodontal pathogens. Thus present study is planned to develop a product from these medicinal plant extract so that oral health of maximum number of people will be improved through herbal preparation.

3.0 Need of the study

Periodontitis is one of the most prevalent disease among all the oral diseases that is confined not only in mouth but also cause occurrence or increase the severity of systemic diseases (WHO, 2011). Periodontitis is one of the causative factor for increasing the risk of non-communicable diseases by increasing the oxidative stress and rise in inflammatory markers (Buhlin et al., 2003). Periodontitis is one of the risk factor left inconsiderate in treatment of some systemic diseases. Main causative factor for prevalence of periodontitis is people's ignorant attitude toward oral health which is less than general health (Aggnur et al., 2014). Thus by controlling the growth of periodontitis causing bacteria not only oral health status will improve but also oxidative stress caused by periodontal bacteria will be reduced thereby general health will also improve (Jeffcoat et al., 2014).

"When all treatment fails, naturopathy can still save a person's life and health. Medicines and operation should be the last treatment not first." – Mahatma Gandhi (1869-1948)

Medicinal plants are natural source of mineral and vitamins and also have active phytoconstituents Due to these active phytoconstituents, plants have medicinal properties. (Luqman et al., 2014). Use of herbal products is more effective and safe. Plant molecules and extracts might be developed into products which possibly will be used by public to treat oral infections. Plants have been found to be safer and effective in curing oral diseases. Herbs have abilities to act on plaque, bacterial inflammations. Before the launching of newer commercial synthetic oral hygiene products, natural plant resources had been used by families for maintaining oral hygiene without side effects (Sharma et al, 2015).

As mentioned in review of literature, recent studies evaluating the effectiveness of these products have shown encouraging results. Use of natural plant products is not only safe but is also economically cheaper and more over easily available. By using herbal extract, the active component of herbs is incorporated into oral hygiene product (Sharma et al, 2015).

Thus, present study is planned to determine the efficacy of selected medicinal plant against periodontal pathogens individually or in different combinations of selected medicinal plants then

individual medicinal plant extract or different combinations which will be found effective during pilot study will be developed in an herbal product for intervention in clinics. If product found to be effective in preventing and curing periodontitis it will be launched for commercial use so that people's not only oral health as well as general health will be improved in a cost-effective manner.

4.0 Statement of the Problem

"Impact of Medicinal Plants Incorporated Product on Patients Suffering from Periodontitis."

5.0 Operational Definitions

Impact-To have a direct effect (Merriam-Webster dictionary, 2003).

For present study impact means, effect of developed product on prevention and cure of periodontitis.

Medicinal Plants- Herbs that are used for therapeutic purpose for prevention or treatment of illness (WHO, 2000).

For present study *Tulsi, Amla, Stevia, Cinnamon* and *Licorice root* will be taken.

Incorporated- To blend or combine thoroughly (Merriam-Webster dictionary, 2003).

Periodontitis –According to **American Academy of Periodontology** "It is chronic inflammation of the tooth supporting structure of tooth i.e. Peridontium" (AAP, 2000)

Product - Something that is made be sold or used ((Merriam-Webster dictionary, 2003).

For present study mouthwash/gel/candy will be developed incorporated with selected Medicinal Plants.

6.0 Objectives of the study

1) To determine the efficacy of phytoconstituent of selected medicinal plants for product development,
2) To develop a product (Mouthwash/Gel/Candy or any other suitable product) incorporated with selected medicinal plant/s (*Tulsi, Amla, Stevia, Cinnamon, licorice root*) by optimizing the efficacy of extract/extracts.
3) Intervention of the developed product for a period of 30 days daily on patients with periodontitis in the Experimental group.

7.0 An Overview of Proposed Research

7.1 Locale of the study - Mathura city and Agra city will be conveniently selected for the study

7.2 Sample selection -

i) Criteria for sample selection -

a) Inclusion criteria

i. Volunteers will be from Mathura and Agra city.
ii. Both male and female will be included.
iii. Male and female between 30-45-year age group.
iv. Patients suffering from mild to moderate periodontitis.
v. Patients, who will be willing to cooperate, give their agreement for the intervention will be selected.

b) Exclusion criteria

I. Patients suffering from debilitating diseases like coronary heart diseases, diabetes, renal diseases, COPD etc.
II. Undergone surgery within 6 months.
III. Taking medications like corticosteroids, lipid lowering drugs, any type of antibiotics or medicinal therapy both (allopathy and naturopathy).
IV. Pregnancy and lactation.
V. Alcoholics & Smokers.

ii) Selection of patients – Volunteers will be selected by the help of dentists in their clinics by asking subjects to fill the modified oral health questionnaire by WHO to

assess the past medical and dental history, oral hygiene habits and as well as dietary habits and then dental checkup will be done by the dentist for confirmation of periodontitis and then a consolidated list of patients with periodontitis will be prepared.

7.3 **Procedure of the study**

Procedure of the study will be

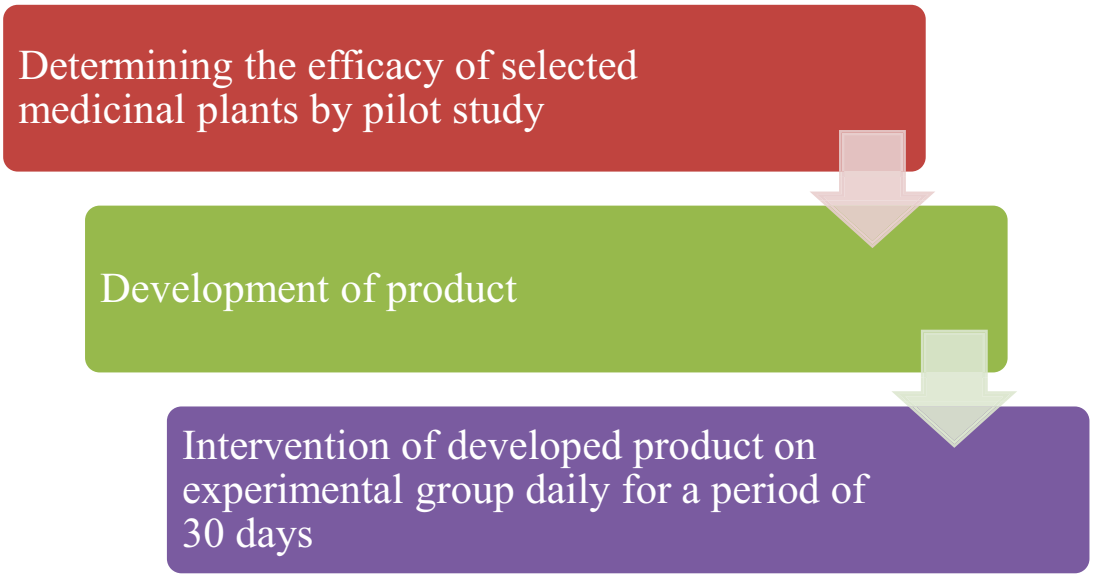
Determining the efficacy of selected medicinal plants by pilot study
Development of product
Intervention of developed product on experimental group daily for a period of 30 days

Figure 7.3 – Procedure of the study

Objective 1 – To determining the efficacy of phytoconstituent of selected medicinal plants for development of product.

A) Preparation of extracts

i) Extract of each of the selected medicinal plants will be prepared.

ii) Combination of extracts from all the above five medicinal plants will be tested in the ratio of 1:1.

a) Preparation of Tulsi extract (Aggarwal et al., 2010)

1) Leaves of Tulsi (Ocimum sanctum) will be obtained from nursey.
2) Leaves will be dried in sunlight or in controlled condition.

3) Dried leaves will be powdered and soaked with 100% ethanol for a week.
4) After one week, filtration will be done through filter paper or muslin cloth.
5) Obtained filter will be reduced at a low temperature < 60 degree Celsius to obtain a solid residue of Tulsi extract.
6) Final concentration of Tulsi extract will be obtained after suspending the extract in polyethylene glycol (20% v/v) and sterile distilled water.
7) Flavoring agent 0.005% spearmint oil may be added to the extract.

b) Preparation of Cinnamon extract

1) Dried bark of cinnamon will be obtained and grinded. Extract will be prepared by any one of following methods –

i) Grounded powder of cinnamon will be suspended in Ethanol (Et OH) using soxhlet apparatus for 6 hours. Then the extract was filtered and evaporated on water bath at 60-80°C (Mariappan et al., 2015).

ii) **Ethanol extract –** Grinded powder of cinnamon will be soaked in ethanol in sterilized bottles with constant agitation (130 rpm) overnight at 20°C in a temperature controlled bio shaker. The ethanol fraction will be separated using sterilized cheesecloth and will be filtered through sterilized Whatman filter paper (No. 2) (Hoque et al., 2008).

iii) **Aqueous extract -** The residual material sample after ethanol extraction will be dried at 40°C, overnight in an oven. Then sterilized distilled water will be added to each dried residue and will be agitated (130 rpm) overnight at 20°C in the temperature controlled bio shaker. The aqueous fraction will be separated by sterilized cheesecloth and sterilized Whatman filter paper (No. 2). extract will be then concentrated using a rotary vacuum evaporator at 40°C, and then concentrated extract will be diluted to 10% Dimethyl sulfoxide as solvent, sterilized by filter (0.45μm), and will be kept at −20°C until use (approximately 2 months) (Hoque et al., 2008).

2) Extraction of essential oils –

i) The essential oils of cinnamon will be extracted using a solvent–solvent extraction method. Ground cinnamon will be added to hexane and will be kept at 20°C in the bio shaker at 150 rpm for 24 h, and then hexane fraction will be separated by squeezing through sterilized cheesecloth. The hexane from the fraction will be evaporated using a vacuum evaporator, which left mass of organics called concrete. Ethanol (99.5 %) will be added to the concrete, and the material will be transferred into a separation funnel, will be vigorously shaken, and then will be kept for several hours for sedimentation of the ethanol insoluble part. The ethanol soluble part will be poured into an evaporator flask and concentrated by vacuum evaporator until all of the ethanol will be completely evaporated, leaving the absolute essential oil (Hoque et al., 2008).

ii) Dried bark of Cinnamon will be obtained and grinded. Coarsely grinded herbal material will be used for extraction of essential oil using the hydro-distillation technique for 8 hours. The oily layers will be separated and dried using anhydrous magnesium sulphate, will be purified through a modified wool filter and will be finally stored at 4°C in air-tight bottles until use (Zainal-Abidin et al., 2013).

c) Preparation of Amla extract (Elobeid & Ahmed, 2015)

i) Amla fruit will be obtained and shade dried.

ii) Dried fruit of Amla will be grinded and soaked in distill water for a 24 hour with a magnetic stirrer at room temperature.

iii) After 24-hour mixture, will be strained using sieve and crude extract will be air-evaporated.

d) Preparation of Liquorice extract (Tian et al., 2008)

i) Dried licorice root will be obtained and grinded.

ii) Dried powder of licorice root will be dipped in ethanol and water mixture for certain period of time at room temperature to 50C. Higher the temperature shorter the dipping time.

iii) The extracts of licorice were separated

e) Preparation of Stevia extract (Afandi et al., 2013).

i) Fresh leaves of Stevia rebaudiana will be obtained, washed and will be spread on trays covered with cheese cloth to remove excess water. The leaves will be also dried in direct sunlight at temperature ranged from 25°C to 30°C for 24 hour to 48 hour.

ii) Then dry leaves will be blended to fine powder.

iii) The grounded fine powder will be extracted with hot methanol using soxhlet apparatus for 2 hours.

iv) The extract will be filtered by using filter paper (Whatmann No. 1) and the residue will be re-extracted two times with methanol.

v) The filtrate will be further concentrated in rotary flash evaporator at 60C.

vi) Little amount of distilled water will be added to dissolve the crude extract.

vii) The extract will be further separated by using separatory funnel with diethyl ether as a reagent to remove the green color.

viii) Lower transparent layer will be collected and extracted again with Butanol.

ix) Finally, the butanol extract (upper layer) will be collected.

B) Check of efficacy of extract (Pilot study)

a. To check the efficacy of the prepared extract intervention of extract will be done on periodontitis patients.

b. Sample size for this objective will be 10 patients for each medicinal plant extract i.e. Tulsi, Cinnamon, Licorice, Stevia, and Amla and also each of different combinations of medicinal plant mix extracts.

c. The patients will be asked to apply the extract/s on their gums with the help of sterilized cotton swab twice per day daily. The patient will be requested not to gargle or put anything in mouth for at least 2 minutes.

d. This procedure will be carried out daily for a total period of 15 days.

e. After 15 days, oral check-up for periodontitis by a dentist will be done.

C) **Completion** of this objective will pave our way on the choice of medicinal plant to see the efficacy on periodontitis (that is which of the medicinal plants/s or a mix of all to be used).

Objective 2 - To develop a product (Mouthwash/Gel/Candy or any other suitable product) incorporated with selected medicinal plants (*Tulsi, Amla, Stevia, Cinnamon, licorice root*).

i) After determining the effectiveness of individual medicinal plant extract or medicinal plant extracts combinations whichever is effective in preventing and curing periodontitis will be selected for product development.

ii) Researcher will aim to develop a product which will be relevant to cure periodontitis like - a Mouthwash/ Gel/ candy or any other suitable product incorporated with the selected medicinal plant/s.

iii) Analysis of nutrient constituent and active phytoconstituent of the developed product will be done.

iv) The product which will have maximum concentration of active phytoconstituent will be selected for intervention programme.

Objective 3 - Intervention of the developed product for a period of one month daily on patients with periodontitis in the Experimental group.

i) Sample selection will be done by above mentioned criteria.

ii) Sample size will be of 25 patients in each experimental and control group that is total 50 patients.

iii) Intervention of developed product will be done daily for 30 days. In case of mouthwash, 2 times rinse after brush, morning and evening for 2 minutes. In case of gel, 2 times light massage on gums after brush and in case of candy, bid dose will be intervene for 30 days.

iv) After 30 days, oral check-up for periodontitis will be done to see the impact of developed product in the experimental group.

v) Comparison will also be done between experimental and control group on completion of 30 days.

Experimental Design

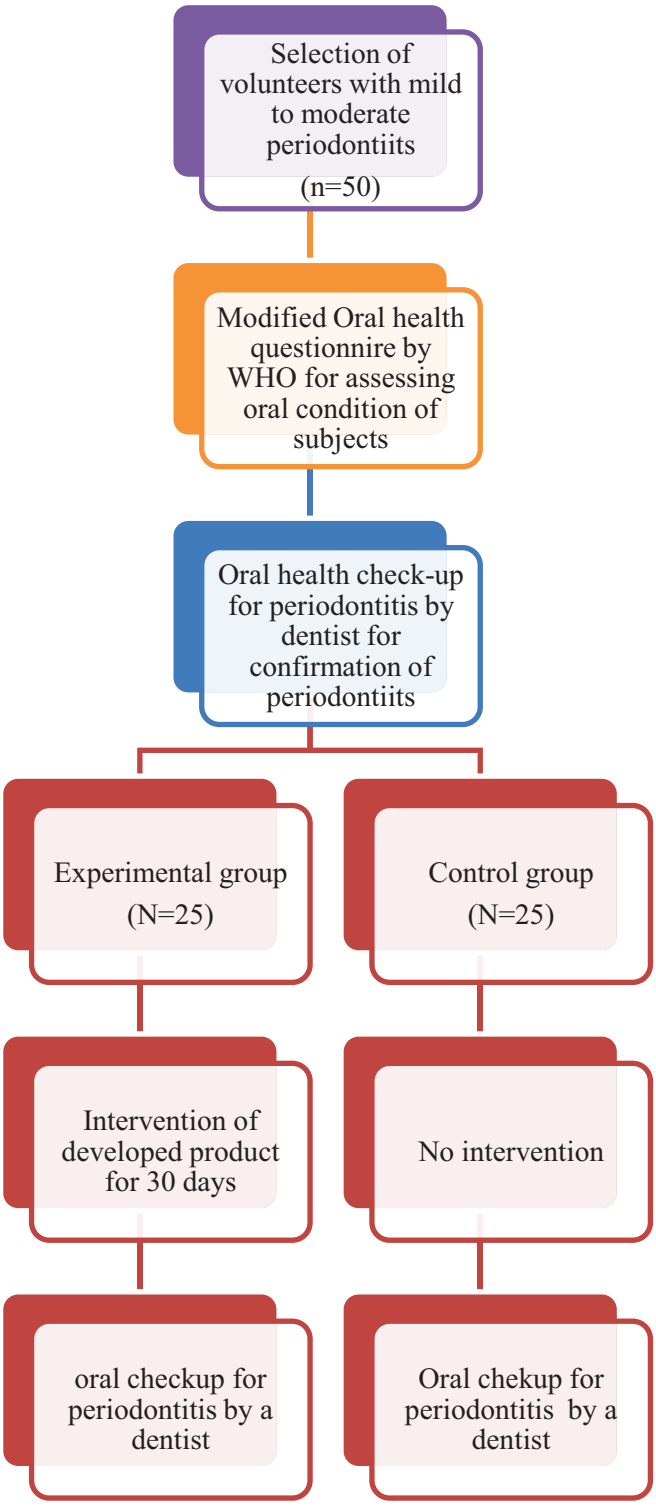
Selection of volunteers with mild to moderate periodontiits (n=50)
Modified Oral health questionnire by WHO for assessing oral condition of subjects
Oral health check-up for periodontitis by dentist for confirmation of periodontiits
Experimental group (N=25)
Control group (N=25)
Intervention of developed product for 30 days
No intervention
oral checkup for periodontitis by a dentist
Oral chekup for periodontitis by a dentist

Figure 7.3 – Experimental design of objective 3

Tools to be used for Present study

i) Questionnaire - Modified Oral health questionnaire for adult given by WHO for assessing their past and present medical and dental history, dietary habits and oral hygiene habits.

Questionnaire will be administered twice during selection of subjects for checking the efficacy of extract and during selection of volunteers for intervention of product.

ii) Assessment tools –Oral checkup by dentist for confirmation of Peridontitis

Statistical analysis

After collecting data of the experiment, relevant test will be applied to see the difference between parameters of pre-and post-intervention. Comparative analysis will be done amongst the various groups and relevant results will be discussed.

Delimitations of the study

- The area of study will be limited to Mathura and Agra city only.
- Study will be limited to see the impact of medicinal plant on cure and prevention of periodontitis only.
- Study includes only five medicinal plants.

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